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Chapter 12

Phosphorylation of PRH/HHEX by Protein Kinase CK2 Regulates Cell Proliferation and Cell Migration in Diverse Cell Types

Padma-Sheela Jayaraman, Kerry S. Wadey, Sarah J. George and Kevin Gaston

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.72902

Abstract

Disruption of the regulatory mechanisms that control cell proliferation and cell migration results in multiple disease states including cancer and leukaemia. The proline-rich homeodomain protein (PRH)/haematopoietically expressed homeobox protein (HHEX) is a transcription factor that controls cell proliferation and cell migration in a variety of tissues in the adult and in the embryo. Phosphorylation of PRH by Protein Kinase CK2 (Casein Kinase II) stops PRH from binding to DNA and regulating the transcription of its direct target genes. In leukaemic cells, phosphorylation also results in the production of a transdominant-negative truncated PRH phosphoprotein by the proteasome. Phosphorylation of PRH is increased in breast and prostate cancer cells and the consequent loss of PRH activity increases cell proliferation and migration. PRH also regulates the proliferation of vascular smooth muscle cells and CK2-dependent phosphorylation of PRH in these cells accompanies increased cell proliferation during intimal thickening. Thus the ability of PRH to regulate cell behaviour and the control of PRH by CK2 is not limited to a specific cell type or tissue. This raises the possibility that the PRH-CK2 axis could be targeted in a variety of disease states ranging from multiple cancers to the intimal thickening that occurs in vein bypass graft failure and restenosis.

Keywords: cell proliferation, cell migration, cell invasion, tumourigenesis, tumour growth, restenosis, intimal thickening

1. Introduction

The proline-rich homeodomain (PRH) or haematopoietically expressed homeobox (HHEX) protein, is a highly conserved transcription factor belonging to the homeodomain family (reviewed by Soufi et al. [1]). Originally characterised in the haematopoietic compartment

[2–4], PRH has since been found in a wide variety of tissues [1]. PRH is critically important in embryonic development where it regulates anteroposterior axis formation and the development of multiple organ systems including the liver, thyroid, lung, thymus, gallbladder and pancreas [5–8]. In the adult, PRH is expressed in a variety of tissues including the thyroid, lungs, liver and haematopoietic compartment [4, 9]. In these tissues PRH acts as a master regulator of genes important in cell proliferation, cell migration and invasion, and cell differentiation [1]. Changes in PRH activity therefore have profound effects on cell behaviour. This review focuses on the regulation of PRH activity by Protein Kinase CK2 and the role that this plays in tumourigenesis and in the control of vascular smooth muscle cell (VSMC) proliferation during intimal thickening.

2. The regulation of gene expression by PRH

2.1. The PRH protein

The PRH protein has three functional domains; a central homeodomain that mediates DNA binding, with N-terminal and C-terminal domains that regulate transcription (**Figure 1**). The PRH homeodomain is a 60 amino acid sequence that forms three α helices. The second and third helices make up a helix-turn-helix motif and together with amino acids in an N-terminal arm of this domain, this mediates sequence-specific DNA binding. The mutation of asparagine to alanine at position 187 within the PRH homeodomain dramatically reduces DNA binding and prevents PRH from repressing the transcription of its direct target genes [10, 11]. The PRH homeodomain also mediates binding to transcription factor AP1 [12]. The PRH N-terminal domain can repress transcription when attached to a heterologous DNA binding domain [10, 13]. Additionally, the N-terminal domain interacts with a variety of proteins including the promyelocytic leukaemic (PML) protein, eukaryotic initiation factor 4E (eIF-4E), proteasome subunit C8, and the regulatory subunit of Protein Kinase CK2

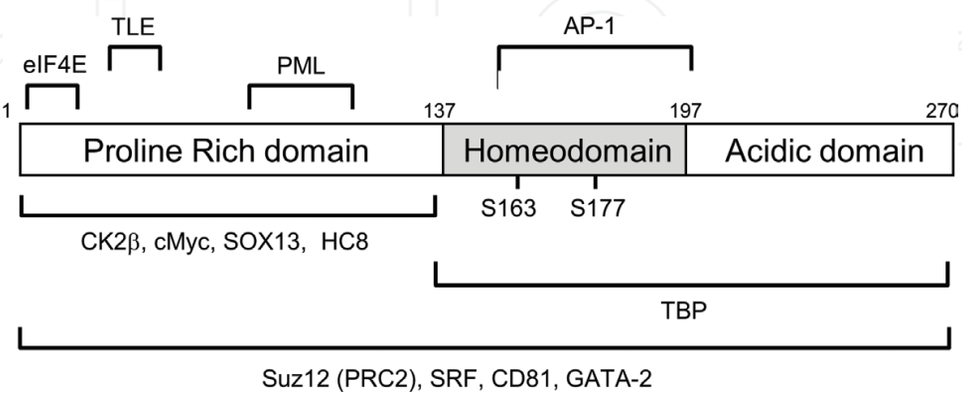

Figure 1. PRH and PRH-interacting proteins. A schematic representation of the PRH protein. The homeodomain and the N- and C-terminal domains are indicated along with the serine residues known to be phosphorylated by CK2 (S163 and S177). PRH-interacting proteins are listed and their binding sites on PRH are indicated by brackets. Some of the protein-protein interactions have not been mapped to defined regions of PRH.

[14–17]. The C-terminal domain is rich in acidic residues and it also functions in transcriptional regulation since its loss prevents PRH from activating transcription of the sodium-dependent bile acid co-transporter (NTCP) gene [18, 19].

The PRH protein forms oligomeric complexes *in vitro* and in cells [20]. The PRH N-terminal domain is resistant to SDS (sodium dodecyl sulphate)-induced denaturation and does not have extensive α-helical or β-sheet secondary structures. However, this domain forms dimers that interact with the PRH homeodomain [20]. *In vitro* studies suggest that octameric PRH oligomers form via the association four PRH dimers [20]. This has implications for DNA binding since although the isolated PRH homeodomain binds to a single DNA site, the full length PRH protein binds to linear arrays of homeodomain binding sites with high affinity [21]. Several genes that are directly regulated by PRH including Goosecoid (GSC), TLE4, VEGFA, VEGFR-1 (FLT1), and endothelial cell-specific molecule-1 (ESM-1) contain multiple, putative PRH-binding sequences [8, 21–23]. This suggests that PRH oligomers bind to these linear arrays to regulate gene expression. However, it is possible that a single PRH binding site may be sufficient to confer gene regulation by PRH.

2.2. The regulation of gene expression

Like many transcription factors PRH can either repress or activate transcription depending on its target gene (see Soufi et al. [1] and Gaston et al. [40] for lists of PRH target genes) and its partner proteins (shown in **Figure 1**). For example, PRH represses the Goosecoid, ESM-1, VEGFA, VEGFR-1, VEGFR-2, and thyroglobulin promoters [8, 21, 23, 24]. An Eh1 motif in PRH N-terminal domain allows PRH to recruit members the Groucho/transducin-like enhancer of split (TLE) family of co-repressor proteins which in turn recruit histone deacetylases [25]. Similarly, PRH can repress transcription by recruiting the polycomb-repressive complex 2 (PRC2) to target genes to bring about histone methylation [26]. These co-repressor interactions can bring about short- and long-range transcriptional repression through histone modification and consequent chromatin condensation. PRH can also repress transcription by interfering with other transcription factors. Binding of PRH to GATA-2 suppresses GATA-2-mediated activation of vascular endothelial growth factor receptor 2 (VEGFR-2) transcription [27]. Similarly, PRH binds to Jun and cMyc inhibiting Jun- and cMyc-dependent transcription activation, respectively [12, 28]. PRH also activates transcription through multiple mechanisms including direct binding to target promoters as in the case of the NTCP promoter [19]. Moreover, PRH binding to hepatocyte nuclear factor 1α (HNF-1α) and serum-response factor (SRF) increases HNF-1α- and SRF-activated transcription [29, 30]. In addition, PRH can regulate gene expression post-transcriptionally through binding to eIF-4E. PRH binding to eIF-4E in PML nuclear bodies disrupts these structures and blocks eIF-4E-dependent transport of cyclin D1 mRNA down-regulating cyclin D1 protein expression [15].

2.3. PRH activity in tumourigenesis

Inappropriate expression and/or aberrant subcellular localization of PRH has been observed in a variety of disease states including acute myelogenous leukaemia (AML) [31, 32], chronic myelogenous leukaemia (CML) [32], breast, thyroid, and prostate cancer [33–36], liver disease,

and cardiovascular disease [37, 38]. In normal haematopoietic cells PRH protein is clearly discernable in distinct foci within the nucleus, co-localising with PML and translation factor eIF-4E [14, 25], whereas in AML and CML PRH appears to be mislocalised to the cytoplasm [32]. Comparably, in thyroid cancer and breast cancer cells, PRH appears to be mislocalised from the nuclear compartment to the cytoplasm and often shows down-regulation in expression [33–35]. In breast and prostate cells loss of PRH activity results in increase cell proliferation and increased cell migration and invasion [35, 36]. Moreover, PRH over-expression in mouse mammary tumour cells inhibits tumour growth *in vivo* [36]. Similarly, PRH over-expression in liver cancer cells inhibits tumour growth in a xenograft mouse model [39] and PRH directly interacts with c-Myc to inhibit hepatocyte proliferation [28]. These studies are consistent with PRH playing a tumour suppressive role in these cell types.

In contrast, PRH has been shown to function as an oncoprotein in T-cell lineages and in AML subtypes (reviewed by Gaston et al. [40]). In retroviral insertion experiments in mice (Lvis1)-elevated PRH expression is associated with B-cell- and T-cell-derived leukaemias and lymphomas [41, 42]. Transgenic mice with ectopic PRH expression in T cell progenitors showed increased numbers of progenitors but this did not result in leukaemia [43]. However mice transplanted with bone marrow cells transduced with a retrovirus expressing PRH exhibit aggressive neoplastic transformation within T-cell populations [44] and in mouse models of early T-cell precursor-like acute lymphoblastic leukaemia (ETP-ALL), PRH is important in Lmo2-driven T-cell self-renewal [45, 46]. Furthermore, in a mouse model of AML elevated PRH is essential for the initiation and maintenance of the leukaemia [26]. Interestingly a human AML has been identified where alteration of the Nup98 and PRH genes to form a fusion gene is the only identified cytogenetic abnormality [31].

2.4. PRH in vascular compartments

PRH is expressed in the developing vascular system in haematopoietic and endothelial progenitor cells [9]. PRH over-expression inhibits haematopoietic and vascular development in embryoid bodies [47] while PRH loss leads to abnormal vasculogenesis and cardiac morphogenesis [5]. PRH can inhibit the proliferation of leukaemic cells by repressing the transcription of VEGFA and other genes involved in VEGF signalling and haematopoietic and vascular biology [48]. PRH is also important in neo-angiogenesis; in endothelial cells PRH represses transcription of multiple genes that control blood vessel formation including VEGFR-1, VEGFR-2, tyrosine kinase with Ig and EGF homology domains (TIE)-1, TIE-2, and neuropilin-1 [27, 49]. PRH is also targeted by urokinase-type plasminogen activator (uPA). uPA regulates angiogenesis and vascular permeability by proteolytic degradation of the extracellular matrix and through intracellular signalling. Single chain uPA is transported from the cell surface receptors to the nucleus where it modulates gene transcription by binding to transcription factors including PRH. The binding of uPA to PRH derepresses VEGFR-1 and VEGFR-2 thereby promoting their expression [50].

Importantly, PRH is up-regulated in VSMCs after balloon injury of the rat aorta [37]. During the period of cell dedifferentiation and cell proliferation following injury, PRH activates transcription of SMemb/NMHC-B, a marker for dedifferentiated cells [37]. Moreover, over-expression of PRH in embryonic fibroblasts results in the expression of early, but not late, markers of VSMC differentiation [29]. It has also been reported that in VSMCs infected with Human Cytomegalovirus (HCMV) PRH up-regulation promotes cell proliferation and

inhibits apoptosis [51]. Our recent work has shown that PRH inhibits the proliferation of human and rat VSMCs (see Section 6.2 [38]). This suggests that HCMV infection may switch PRH from being an inhibitor of VSMC proliferation to an activator.

3. Protein kinase CK2

3.1. CK2 structure

Protein Kinase CK2 (formerly known as Casein Kinase II) is a ubiquitously expressed enzyme important in a range of cellular functions and processes including cell cycle progression and cell migration and invasion [52]. CK2 is a Ser/Thr kinase with the minimal consensus target sequence Ser/Thr– X – X – Asp/Glu/pSer (where X indicates any non-basic amino acid). However, CK2 can phosphorylate wide variety of target sequences. CK2 exists as a hetero-tetrameric enzyme consisting of two catalytic α subunits and two regulatory β subunits. In humans, two isoenzymic forms of the catalytic subunit, designated α and α', are well-characterised while a more recently discovered α'' subunit is less well understood [53–55].

3.2. CK2 function

CK2 is important in the control of cell migration and cell proliferation and in many other cell functions. To this end CK2 is pleiotropic, in that it has multiple effects via the phosphorylation of numerous cytoplasmic and nuclear proteins. For example, phosphorylation of inhibitor of kappa B (IκB) by CK2 causes disassembly of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)-IκB complex [56]. This allows NF-κB to regulate the transcription of genes involved in cell cycle progression and cell survival. CK2 is also important in the control of extracellular proteins. For example, phosphorylation of the extracellular matrix protein vitronectin by CK2 is important for the adhesion of VSMCs [57]. CK2 itself is regulated by multiple signalling cascades and can cross talk to co-ordinate cell survival and cell proliferation. The ABL, Src and ERK kinase families all act as upstream regulators of CK2 and inhibitors that target these kinases can be used to inhibit CK2 indirectly [58–60].

3.3. CK2 in tumourigenesis

Aberrant CK2 activity has been demonstrated to be oncogenic and elevated CK2 expression is seen in multiple cancers including breast [61], prostate [62], lung [63], head and neck [64], and kidney cancers [65]. CK2-mediated abrogation of tumour suppressor activity or stimulation of oncogenic proteins has been demonstrated to play a significant role in tumourigenesis. The tumour suppressors promyelocytic leukaemia protein (PML), connexin, and phosphatase and tensin homology protein (PTEN) are all CK2 substrates that are inactivated by phosphorylation [66]. CK2 has additionally been shown to potentiate aberrant activation of oncoproteins including NF-κB [56], and AKT [67]. Drugs that inhibit CK2 have proven to be well-tolerated in a number of clinical trials and systemic or local delivery of these inhibitors is therefore a potential treatment for multiple disease states [68, 69].

4. The regulation of PRH by CK2

4.1. CK2 binds to PRH and phosphorylates the homeodomain

To identify PRH binding proteins we performed a yeast two hybrid screen using PRH as bait. This showed that the regulatory β subunit of CK2 can bind to the PRH N terminal domain [17]. The interaction was confirmed in human chronic myeloid leukaemia K562 cells using pull-down experiments and co-immunoprecipitation [17]. Importantly, PRH is a phosphoprotein in these cells and pharmacological inhibition of CK2 with DMAT (2-dimethylamino-4,5,6,7-tetrabromo-1H-benzimidazole) or TBB (4,5,6,7-tetrabromo-1H-benzotriazole) significantly reduces the amount of phosphorylated PRH (pPRH) indicating that PRH is also a CK2 substrate [17]. CK2β controls substrate specificity and therefore the interaction with PRH is potentially of importance for the control of CK2 activity on other specific substrates as well as in the phosphorylation of PRH.

To map CK2 phosphorylation sites within PRH, purified, human PRH protein was incubated with CK2 and ATP and subjected to surface-enhancer laser desorption/ionisation time-of-flight mass spectrophotometry (SELDI-TOF-MS) analysis. This showed that S163 and S177 located within the PRH homeodomain can be phosphorylated by CK2 [17]. S163 is located within a CK2 target consensus site while S177 is within a non-consensus site. Subsequently further phosphorylation sites have been identified within PRH but these sites have not been associated with a specific kinase.

4.2. Phosphorylation of PRH blocks DNA binding

Phosphorylation of the PRH homeodomain by CK2 abrogates PRH DNA-binding activity *in vitro* [17]. Interestingly DNA binding activity is restored by a subsequent incubation of pPRH with calf intestinal alkaline phosphatase. Thus, CK2-mediated phosphorylation of PRH functions as a reversible switch for DNA binding [17]. CK2 has also been shown to inhibit the binding of PRH to DNA in cells. Ectopic over-expression of PRH in K562 cells represses transcription of the PRH target gene VEGFR-1 but this repression is lost on co-transfection with CK2α and β transgenes [48]. However, the repression of VEGFR-1 transcription by a PRH mutant in which phosphorylation of serine 163 and serine 177 is prevented by the replacement of these residues by cysteine residues is not inhibited by CK2 over-expression [48]. Quantitative chromatin immunoprecipitation (ChIP) showed that CK2 over-expression does not prevent the binding of PRH S163C,S177C to the VEGFR-1 promoter as it does with wild-type PRH [48].

4.3. Phosphorylation of PRH induces protein processing

Hypo-phosphorylated PRH is stable in K562 cells treated with the translation inhibitor anisomycin [48]. However, pPRH is rapidly degraded in these cells. The half-life of pPRH is extended by treatment with proteasome inhibitors showing that phosphorylation targets PRH for proteasome-mediated protein cleavage. Interestingly, pPRH is cleaved to produce is a stable truncated protein that lacks the C-terminal domain (PRHδC). Over-expression of

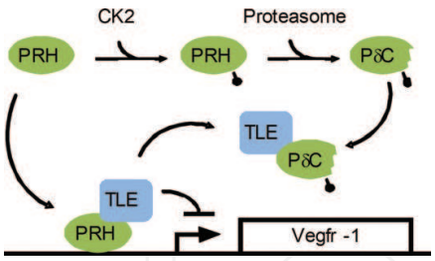

Figure 2. Phosphorylation of PRH by CK2 induces protein cleavage. PRH recruits co-repressor proteins including TLE to target genes such as VEGFR-1 in order to repress transcription. Phosphorylation of PRH by CK2 (shown as a filled lollipop) prevents PRH from binding to DNA and targets the protein for processing by the proteasome. The PRHδC protein cannot bind to DNA but it can sequester TLE proteins (and possibly other PRH interacting proteins) and thereby block transcriptional repression by PRH.

CK2 increases the production of this cleavage product and the truncated protein can act as transdominant negative regulator of full-length PRH by sequestering TLE co-repressor proteins and possibly other PRH interacting proteins [48]. This suggests that phosphorylation of PRH not only blocks DNA binding but also acts to prevent unphosphorylated PRH from regulating transcription (**Figure 2**). As might be expected, PRH S163C,S177C cannot be phosphorylated at these residues and this protein is not processed by the proteasome. In contrast, the phosphomimetic PRH S163E,S177E is more readily processed to produce PRHδC than wild type PRH [48].

4.4. pPRH in tumourigenesis

Pre-clinical studies have shown that pPRH is elevated in benign prostatic hyperplasias and in breast ductal carcinoma *in situ* compared to normal tissues [35, 36]. PRH localization is also altered in prostate and breast tumours compared to normal tissue. Both increased pPRH and increased PRH cytoplasmic localization are indicative of PRH inactivation and it is likely that this contributes to increased cell proliferation in these diseases. Interestingly, pPRH is less highly elevated in aggressive prostate adenocarcinomas and invasive breast carcinomas [35]. This could be due to decreased total PRH expression in these cancers. Thus high levels of pPRH appear to correlate more with hyperproliferative disease in these tissues rather than with advanced cancer.

5. PRH and CK2 in tumourigenesis

5.1. pPRH and PRH as potential biomarkers

The identification of protein modifications that contribute to increased cancer cell proliferation and increased cell migration and invasion is likely to result in new therapeutic approaches that could be of great benefit to patients. Moreover such cancer biomarkers could be useful as prognostic indicators and as indicators of pharmacologic responses to a therapeutic intervention. Prognostic biomarkers that can flag a tumour as potentially benign or requiring further treatment are urgently required. Many breast and prostate tumours for example do not need

intervention and are currently over-treated by surgery because of a lack of biomarkers for prognosis. In pre-clinical studies the levels and localization of pPRH and PRH appear to be altered in breast and prostate tumours compared to controls [35, 36]. However, additional studies with large numbers of patients will be required to determine whether pPRH and PRH or the pPRH/PRH ratio is a good prognostic indicator.

5.2. The restoration of PRH function

Since PRH appears to be inactivated in breast and prostate cancer cells by CK2-dependent phosphorylation resulting in increased cell proliferation and cell migration, the inhibition of CK2 in these tissues would be expected to restore PRH function. This would be expected to inhibit cell proliferation and it could inhibit tumour growth. CK2 inhibitors have been proposed as novel treatments for multiple cancers including prostate cancer. In normal immortalised prostate epithelial cells the inhibition of proliferation brought about by the inhibition of CK2 requires the presence of PRH [35]. It is likely that CK2 inhibitors will have similar effects in other cancer cell types through the prevention of PRH phosphorylation and the restoration of PRH function. Indirect inhibition of CK2 activity can also restore PRH function and re-establish the control of cell proliferation. Our previous work showed that in chronic myeloid leukaemia cells Dasatinib decreases CK2 activity and decreases the phosphorylation of PRH [58]. Dasatinib inhibits membrane bound tyrosine kinases and Src family kinases and is an efficacious therapeutic for leukaemias expressing BCR-ABL fusion proteins and those with activated Src [70]. Importantly, Src-kinases are known to stimulate CK2 activity [59]. It is possible that other Abl/Src kinase inhibitors will also restore PRH activity via the indirect inhibition of CK2. However, since PRH can act as oncoprotein in some cell types it is possible that the reduction of PRH phosphorylation in these cell types might be counterproductive.

6. Saphenous vein graft failure

6.1. Intimal thickening in saphenous vein grafts

Atherosclerotic plaque development within coronary arteries is a major precursor for myocardial infarction (commonly known as heart attack). Coronary artery bypass graft (CABG) surgery is an effective treatment for occlusive or ruptured coronary artery atherosclerotic plaques; surgery most often involves harvesting and grafting of healthy, autologous saphenous vein to bypass the occluded artery and facilitate revascularisation of the cardiac tissue [71, 72]. Arteriovenous grafts are however predisposed to reblocking (restenosis), and despite extensive research, 10–15% of CABG patients suffer early vein graft failure within the first year after surgery, and as many as 50% suffer graft failure within 10 years [71–73]. Thrombosis, intimal thickening, and accelerated atherosclerosis are the underlying causes of saphenous vein graft failure. Intimal thickening, which serves as a foundation for superimposed atherosclerosis, is often the cause of late vein graft failure (**Figure 3**), while thrombosis is the cause of early graft failure. Intimal thickening is a product of aberrant VSMC migration into the intima where they proliferate and deposit extracellular matrix.

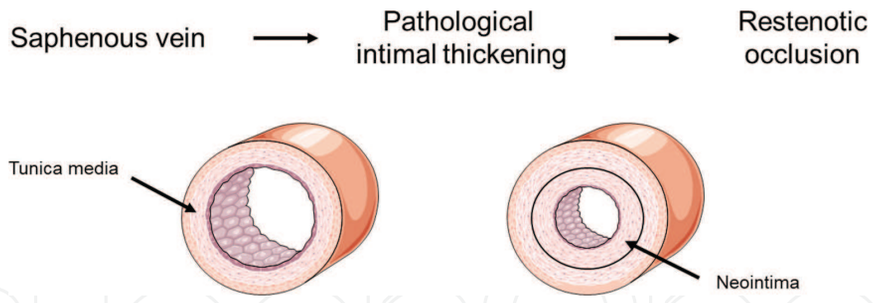

Figure 3. Intimal hyperplasia in saphenous vein grafts. Intimal hyperplasia in saphenous vein grafts is a consequence of the migration of medial VSMCs to the intima and their subsequent proliferation and deposition of extracellular matrix. Neointima formation results in narrowing of the lumen and a consequent restriction of blood flow.

6.2. PRH is up-regulated in neointimal cells

PRH expression is up-regulated in the intimal compartment of rat thoracic aortas injured with a balloon embolectomy catheter – a robust model for neointimal hyperplasia [37]. However, PRH mRNA and protein expression is absent in healthy aorta. Moreover PRH activates transcription of SMemb/NMHC-B, a marker of dedifferentiated VSMCs with a synthetic, proliferative phenotype, and not of differentiated VSMCs with a quiescent, contractile phenotype [37]. Together these findings could indicate that PRH promotes VSMC de-differentiation and accumulation in the intima, thereby accelerating disease progression. However, ectopic overexpression of wild-type PRH in primary cultures of rat aortic VSMCs inhibits cell cycle progression, whereas siRNA-mediated knockdown of PRH promotes cell proliferation [38]. These data clearly indicate an anti-proliferative role for PRH in VSMCs. Transfection of isolated rat aortic VSMCs with a vector expressing PRH F32E, a mutant that does not bind TLE, did not block cell proliferation suggesting that in these cells, PRH inhibits cell cycle progression in a TLE-independent manner (KSW unpublished observations). Interestingly, PRH S163C,S177C exhibited a prolonged anti-mitotic effect with respect to wild-type PRH [38]. This indicates that phosphorylation of PRH at Ser163 and Ser177 prevents PRH from inhibiting VSMC proliferation. Moreover, adenovirus-mediated gene transfer of PRH S163C,S177C retarded intimal thickening in an *ex vivo* human saphenous vein organ culture model [38]. It is hence likely that PRH is up-regulated during neointima formation in dedifferentiated, proliferating VSMCs as a negative feedback mechanism to prevent further rounds of mitosis.

6.3. CK2 activity during intimal thickening

Multiple studies have implicated the involvement of CK2 in the regulation of VSMC proliferation and pathophysiological intimal thickening. For example, treatment of cultured human aortic smooth muscle cells with emodin (1,3,8-trihydroxy-6-methylanthraquinone) – a naturally occurring CK2 inhibitor used in traditional Chinese medicine – blocked platelet-derived growth factor (PDGF)- and tumour necrosis factor α (TNF-α)-induced cell proliferation in a dose-dependent manner [74]. Also, in the rat aortic VSMC line A10, inhibition of CK2 with the synthetic compounds DDZ (daidzein) and DRB (5,6-dichlorobenzimidazole riboside) antagonised lysophosphatidic acid-induced cell division [75]. However, emodin, DDZ and DRB

show high promiscuity as inhibitors [76]. PDGF, basic fibroblast growth factor (bFGF), and Wnt proteins are well-recognised atherogenic mitogens that are up-regulated in atherosclerotic and restenotic lesions ([38] and references therein). Interestingly, pharmacological inhibition of CK2 with the highly selective compounds TBB and K66 suppresses PDGF-, bFGF- and Wnt-4-induced cell replication in primary cultures of rat aortic VSMCs [38]. Silencing of CK2 using exogenous siRNAs also inhibited VSMC proliferation further suggesting that CK2 promotes the proliferation of these cells. Furthermore, treatment of human saphenous vein organ cultures with the CK2 inhibitor K66 disrupted neointima formation [38].

6.4. CK2 acts via PRH to modulate VSMC proliferation

One mechanism through which CK2 may facilitate VSMC proliferation could be via blocking PRH activity. Treatment with the K66 failed to arrest PDGF- and bFGF-stimulated cell cycle progression in VSMCs with depleted levels of PRH [38]. Thus CK2-dependent mitogenic signal transduction at least in part requires the presence of PRH (**Figure 4**). Similarly, treatment of human immortalised myelogenous K562 cells with the CK2 inhibitor DMAT inhibits cell proliferation but has no significant effect on the proliferation of K562 cells in which PRH has been knocked down using shRNA [58]. In K562 cells PRH controls cell proliferation via the inhibition of VEGF signalling [24, 58]. Further work is required to determine whether PRH controls VSMC proliferation via the inhibition of VEGF signalling or whether other signalling pathways targeted by PRH are important in this context. For instance, another potential mechanism by which PRH might control VSMC proliferation involves urokinase-type plasminogen activator (uPA)-mediated signalling [77]. uPA is a serine protease that is up-regulated in atheromas and restenotic lesions of human arteries [77–79]. In uPA deficient mice, subsequent to either electrical or mechanical arterial injury, intimal thickening and cell accumulation is significantly reduced compared to wild-type mice [80]. In human umbilical vein VSMCs, endogenous uPA has been shown to be involved in the induction of a mitogenic response by either PDGF or bFGF [77]. Furthermore, in PDGF- or bFGF-stimulated cells, pharmacological inhibition of uPA and CK2 with p-aminobenzamidine and 4 μM TBB, respectively, markedly enhances the anti-proliferative effects of 4 μM TBB alone in an additive manner [77]. Intracellular uPA has recently been shown to bind to PRH in endothelial

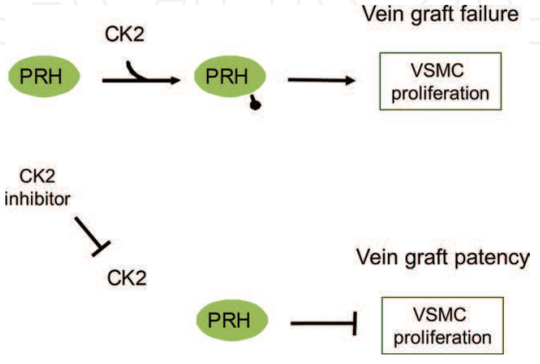

Figure 4. The inhibition of CK2 prevents intimal thickening. Top – phosphorylation of PRH by CK2 prevents PRH from inhibiting VSMC proliferation and this contributes to vein graft failure. Bottom – pharmacological inhibition of CK2 allows PRH to suppress VSMC proliferation and thereby prevent neointima formation. Other CK2 target proteins are also likely to play a role in the prevention of intimal thickening following CK2 inhibition.

cells and to prevent PRH from repressing VEGF signalling genes [50]. Therefore it is possible that the effects of uPA inhibition on endothelial proliferation and CK2 inhibition on VSMC proliferation during intimal thickening are both mediated by PRH.

6.5. Implications for saphenous vein grafts

As protein kinase CK2 is ubiquitously expressed, systemic delivery of a CK2 inhibitor for the treatment of saphenous vein graft degeneration may cause unwanted side effects. However, perivascular drug delivery systems could be employed for localised, sustained release of a CK2 inhibitor to a grafted vein. Such a system has been used to deliver sunitinib in a biocompatible hyaluronic acid-based hydrogel within a polyactide-co-glycolide perivascular wrap [81]. Other approaches for delivery include drug-eluting nanoparticles and drug-linked antibodies [82].

Gene therapy also has therapeutic potential in alleviating saphenous vein graft stenosis, and could be used for the introduction of PRH, particularly PRH S163C,S177C, to grafted vein. Genetic manipulation of a venous graft must however occur peri-operatively, meaning there is only a single opportunity to complete gene transfer. Therefore, helper-dependent adenovirus technology may be necessary to provide prolonged expression of PRH or PRH S163C,S177C within the grafted conduit [83, 84]. In a similar instance, delivery of tissue inhibitor of metalloproteinase 3 (TIMP-3) has been shown to block neointima formation in autologous porcine arteriovenous interposition grafts for up to 3 months [85].

7. Conclusion

The regulation of cell proliferation and cell migration/invasion by PRH is not limited to a particular cell type. Similarly, the control of PRH by CK2-dependent phosphorylation is also seen in multiple cell types. The PRH-CK2 axis is likely to be important for the regulation of cell proliferation and cell behaviour across a broad spectrum of cell types and in a variety of disease states. Further work in this area is therefore likely to be of great clinical relevance.

Acknowledgements

We are grateful to the British Heart Foundation (FS/10/58/28515) and the Medical Research Council for supporting this work. We apologise to the many authors whose papers we have not cited here due to space constraints.

Abbreviations

ATP	adenosine triphosphate
bFGF	basic fibroblast growth factor
CABG	coronary artery bypass graft

CK2	protein kinase CK2 (Casein Kinase II)
DDZ	daidzein
DMAT	2-dimethylamino-4,5,6,7-tetrabromo-1H-benzimidazole)
DRB	5,6-dichlorobenzimidazole riboside
K66	1-carboxymethyl-2-dimethylamino-4,5,6,7-tetrabromo-benzimidazole
PDGF	platelet-derived growth factor
SDS	sodium dodecyl sulphate
SELDI-TOF-MS	surface-enhancer laser desorption/ionisation time-of-flight mass spectrophotometry
TBB	4,5,6,7-tetrabromo-1H-benzotriazole
VSMC	vascular smooth muscle cell

Author details

Padma-Sheela Jayaraman[1], Kerry S. Wadey[2], Sarah J. George[2] and Kevin Gaston[3]*

*Address all correspondence to: kevin.gaston@bristol.ac.uk

1 Institute of Cancer and Genome Biology, College of Medical and Dental Sciences, University of Birmingham, Birmingham, UK

2 Bristol Medical School, University of Bristol, Bristol Royal Infirmary, Bristol, UK

3 School of Biochemistry, University of Bristol, Bristol, UK

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